SCIENTIFIC ABSTRACT

This amended protocol (NIH Protocol 95-I-0135, NIH Biosafety RD-94-XI-05, FDA BB-IND-6100, RAC/ORDA 9503-104) is a Phase I/II clinical trial to determine the efficacy and safety of a method of *ex vivo* gene therapy to treat both X-linked gp91^{phox} deficient Chronic Granulomatous Disease (CGD) and autosomal recessive p47^{phox} deficient CGD. CGD is an inherited immune deficiency in which blood neutrophils and monocytes fail to produce superoxide and other antimicrobial oxidants, and patients get recurrent life-threatening infections. 30 CGD patients of either sex at least 5 years of age may be enrolled in addition to the 5 patients enrolled in the first phase of this trial. Patients less than 16 years of age must have an active infection or a recent relapse of infection at the time of enrollment. Patients may receive up to 6 cycles of gene therapy at intervals of 4 weeks or longer. For the first 3 cycles, a cycle of gene therapy will begin with 8 daily subcutaneous injections of the combination of flt3-ligand (flt3L) at 50 μg/kg/day plus granulocyte-macrophage colony stimulating factor (GM-CSF) at 5 μg/kg/day for mobilization of CD34+ cells. For subsequent cycles (cycles 4-6) a cycle of gene therapy will begin with 6 daily injections of granulocyte colony stimulating factor (G-CSF) at 10 μg/kg/day for mobilization of CD34+ cells. On the last two days of marrow growth factor administration for mobilization of CD34+ cells, the patients will have an apheresis procedure to harvest blood mononuclear cells. CD34+ progenitors will be selected from the apheresis collection using the Isolex® 300i anti-CD34 monoclonal antibody/magnetic bead selection system. CD34+ cells will be cultured in PL2417 gas permeable plastic containers that have been pre-coated with fibronectin fragment CH-296. The growth medium will be serum-free X-VIVO 10® supplemented with 1% human serum albumin, 100 ng/ml flt3-ligand, 50 ng/ml PIXY321 and 50 ng/ml stem cell factor (SCF). Cultured CD34+ progenitors will be transduced on each of 3 or 4 days with either MFGS-p47^{phox} or MFGS-gp91^{phox} retrovirus vectors. The retrovirus vectors are replication defective packaged in amphotropic envelope lines engineered with the CRIP packaging elements (5'LTR-gag-pol-3'SV40polyA; 5'LTR-AMenv-3'SV40polyA). MFGS-p47^{phox} is packaged in the murine ψ-crip line, while MFGS-gp91^{phox} is packaged in the human 293-SPA line. The clinical retrovirus vector supernate will be animal protein-free and serum free. Transduced CD34+ cells will be infused into the CGD patient, completing one cycle of therapy. Safety testing for endotoxin, sterility, and absence of replication competent retrovirus will be performed on the retrovirus producer lines, virus particle lots, and transduced cells. Blood will be tested periodically for the appearance and persistence of neutrophils that are functionally corrected by the gene therapy. The efficacy goal for this study is to allow CGD patients to produce autologous gene-corrected functionally normal NADPH oxidase positive neutrophils to a level of at least 1 in 1000 circulating neutrophils for at least four weeks. This may provide clinical benefit in the form of increased host defense against an ongoing or potential infection. The clinical status of patients will be monitored for any evidence of toxicity. Information obtained from this study will provide information important for achieving the ultimate goal of the development of gene therapy for CGD that will be a permanent cure for this disorder.